Incorporation of thymol into corncob granules for reduction of odor and pathogens in feedlot cattle waste[1,2]

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ABSTRACT: Confined animal feeding operations can be a source of odor emissions, global warming gases, water pollution, and food contamination. Laboratory studies have indicated that plant oils with antimicrobial activity can be used to control pathogens and odor emissions from cattle and swine wastes. However, these oils are aromatic and may volatilize when applied topically. Our objectives were to evaluate the volatility of thymol from a feedlot surface and the effectiveness of topically applying thyme oil (2.5% thymol), incorporated into corncob granules and added once per week, to control odor emissions and total coliforms in feedlot manure. In the first study, thymol either volatilized or was degraded within 28 d after topical application. In a second study, thyme oil (2.5% thymol) was incorporated into corncobs and applied to pen surfaces weekly. Manure samples from 6 locations in each pen were collected from 3 untreated and 3 thymol-corncob-treated pens (15 × 150 m; fifty 400-kg cattle/pen), 3 times per week for 8 wk. Samples were analyzed for thymol concentration, total VFA, branched-chain VFA, aromatic compounds, and the number of *Escherichia coli* and total coliform bacteria. Over the 8 wk, with the exception of wk 7, the desired thymol concentration of 15 to 20 μmol/g DM was maintained in the manure. Concentrations of VFA and branched chain-VFA increased over time in untreated and treated pens. However, the rate of VFA accumulation in treated pens (7.5 ± 1.3 μmol·g DM^{-1}·wk^{-1}) was less ($P < 0.01$) than the rate of accumulation in untreated pens (18.0 ± 2.1 μmol·g DM^{-1}·wk^{-1}). Likewise, the rate of branched-chain VFA accumulation in treated pens (0.31 ± 0.04 μmol·g DM^{-1}·wk^{-1}) was less ($P < 0.01$) than in untreated pens (0.55 ± 0.06 μmol·g DM^{-1}·wk^{1}). The concentrations of *E. coli* in treated pens ($2.9 \pm 1.2 \times 10^5$ cfu·g DM^{-1}) were 91% less ($P < 0.04$) than in untreated pens ($31.1 \pm 4.0 \times 10^5$ cfu·g DM^{-1}). Similarly, concentrations of coliforms in treated pens ($3.7 \pm 1.3 \times 10^5$ cfu·g DM^{-1}) were 89% less ($P < 0.04$) than those of untreated pens ($35.3 \pm 4.2 \times 10^5$ cfu·g DM^{-1}). These results indicate that odor emissions and total coliforms can be reduced in feedlot manure with a once per week application of thymol incorporated in a granular form. However, corncobs are bulky, and other granular carriers with a greater carrying capacity for thyme oil should be explored.

Key words: feedlot manure, odor, pathogen, plant oil

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INTRODUCTION

Elder et al. (2000) reported 28% of feedlot cattle may be carriers of *Escherichia coli* O157 in their feces, and 11% of cattle presented for slaughter carry *E. coli* O157 on their hides. Keen and Elder (2002) found these numbers to be even higher than reported in their first study and observed a correlation between fecal prevalence and carcass contamination. Their data indicate that proper waste management may help control pathogens on the farm. Diez-Gonzalez et al. (2000) reached a similar conclusion with dairy cattle manure. Studies indicate that carbonate-alkali treatment can be an effective mechanism for eliminating *E. coli* from cattle manure slurries (Arthurs et al., 2001; Jarvis et al., 2001). Laboratory studies with the plant-derived oils, carvacrol, thymol, and eugenol, indicate that these antimicrobial agents are effective in eliminating total coliforms and inhibiting odor emissions from cattle and swine waste slurries (Varel and Miller, 2001; Varel, 2002; Varel and Miller, 2004). These chemicals, like most plant oils, are generally recognized as safe. In practice, carvacrol is added to different food products such as baked goods (16 ppm), nonalcoholic beverages (28 ppm), and chewing gum (8 ppm; Ultee et al., 1999). Carvacrol (300 ppm)

[1]Technical assistance of S. Wise, preparation of corncob granules by EcoSMART Technologies, and secretarial assistance by D. Brown and J. Byrkit are appreciated.

[2]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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is also being evaluated as a preservative for rice (Ultee et al., 2000). Thymol is a component in many different products including soaps, toothpastes, shampoos, deodorants, and mouthwashes (Shapiro et al., 1994; Manou et al., 1998). The objectives of our studies were to determine the disappearance or volatility of thymol when topically applied to a feedlot surface and to evaluate thyme oil (2.5% thymol), incorporated into a slow-release granule, for its ability to control total coliforms and odor emissions from feedlot manure.

MATERIALS AND METHODS

Treatment and Manure Sampling

Two studies were conducted to determine the volatility and effectiveness of topically applying thymol to feedlot pens to control total coliforms and odor. An initial experiment was conducted to determine the disappearance or volatility of thymol on the feedlot surface without cattle present. This study was conducted without cattle in the pens in order to avoid a dilution factor from new manure being added to the pens. The finishing diet fed to the approximately fifty 400-kg cattle before they were removed from the pens (1 mo in pens) contained 83% corn, 13% alfalfa hay, and a 4.5% liquid supplement. The supplement was 75% CP and included a nonprotein nitrogen content of 71% (of the CP), 11% Ca, 1% P, 70,000 IU of vitamin A/kg, 152 IU of vitamin E/kg, and 0.306 g of monensin/kg (Elanco Animal Health, Greenfield, IN). Cattle were fed 9.7 kg of DM/steer daily. Behind each feed trough, which extended the width of the pen, was a concrete apron (3.5 m in length). The area adjacent to the apron (1.2 × 15 m; soil base) was used as the experimental area. Efforts were made to topically apply approximately 50, 75, and 100 m*M* of thymol (thymol crystals dissolved in 95% ethanol; Ungerer and Company, Lincoln Park, NJ) to surface manure 1 d after the cattle were removed. Each of 6 pens was divided in half, and one-half (7.5 m) of each pen was treated with thymol using a pressurized hand-held sprayer. The other half of each pen simply received an equivalent amount of ethanol. Each concentration of thymol was duplicated in a second pen. Each treatment unit was sampled from the loose surface at 0, 1, 7, and 28 d, from 6 replicate evenly spaced sites within a pen (50 g/site, which were pooled) and analyzed for thymol as indicated in the sample analyses section of this paper. During this period (June-July) the average daytime temperature was between 27 and 33°C, and the manure DM was 75 to 85%.

A second experiment was conducted the following year once a method was developed to add thymol into a granule. This was done by using thyme oil (2.5% thymol) and incorporating it into ground corncobs (2 to 4 mm) at a 6% concentration of thyme oil (EcoSmart Technologies, Inc., Franklin, TN). A preliminary laboratory study was conducted with the corncob granules to ensure the thymol was released from the granules. This involved methods previously described (Varel and Miller, 2001) in which cattle waste (50:35:15 feces:urine:water) was mixed with either no additions (control), thymol (20 m*M* from crystals), or thymol (20 m*M* from thyme oil) incorporated into corncobs. Waste slurry was divided into 500-mL aliquots, and thymol was added directly to the slurry at the desired concentration. The slurry was blended for 1 min to provide a homogeneous mixture, and poured into 1.6-L jars. Plastic lids provided with the jars were used to cover approximately 90% of the jar to prevent moisture loss over the 50-d experimental period. Production of VFA was used as a measure to determine effectiveness of the thymol from the corncob granules. These treatments were in triplicate. After this preliminary study demonstrated that thymol was released from the granules, a feedlot study was conducted.

Six feedlot pens (similar, but not the same pens described in the first experiment) were used. Every other pen served as an untreated control, and the remaining 3 pens were treated with the thymol-corncob granules. Each pen contained 50 steers (average BW of 400 kg) fed the finishing diet indicated earlier. The treated area in the pen was again the 1.2 × 15 m surface adjacent to the concrete apron. Initially, a Scotts Speedy Green 1000 rotary fertilizer spreader (Scotts Company, Marysville, OH) was used to apply the granules. However, once the DM of the manure was less than 60%, this was impractical, and a hand-held scoop was used. The granules were applied once per week over an 8-wk period with an objective of maintaining 15 to 20 μmol of thymol/g of DM in the waste. In laboratory studies, this concentration was effective at eradicating culturable coliforms and reducing odor (Varel and Miller, 2001). Each pen was sampled 3 times per week from 6 evenly spaced sites (100 g/site) across the 15-m treatment area. For each day, the 6 replicate samples were pooled. This pooled sample was processed as indicated in the sample analyses section of this paper. During this 8-wk period (April-June), the average daytime temperature was between 18 and 28°C, and manure DM was between 30 and 80%.

Sample Analyses

Immediately after collection of 50-g samples (six 50-g samples pooled) in the initial experiment, 15 g was dried at 105°C overnight to determine DM, and 15 g was acidified with 15 mL of 0.5 *M* H_2SO_4 and stored at –20° until analyzed for thymol. Briefly, an 0.5-mL aliquot of the acidified sample was combined with an internal standard, ethyl butyrate (0.25 m*M* final concentration). The sample was acidified with 0.4 mL of 3 *M* HCl; then 0.8 mL of ethyl ether was added and the sample was shaken vigorously for 1 min and centrifuged at 5°C, 16,000 × *g* for 1 min. The ether extraction was repeated a second time, and the ether phase was analyzed for thymol with a Hewlett Packard 6890 gas chromatograph (Varel, 2002).

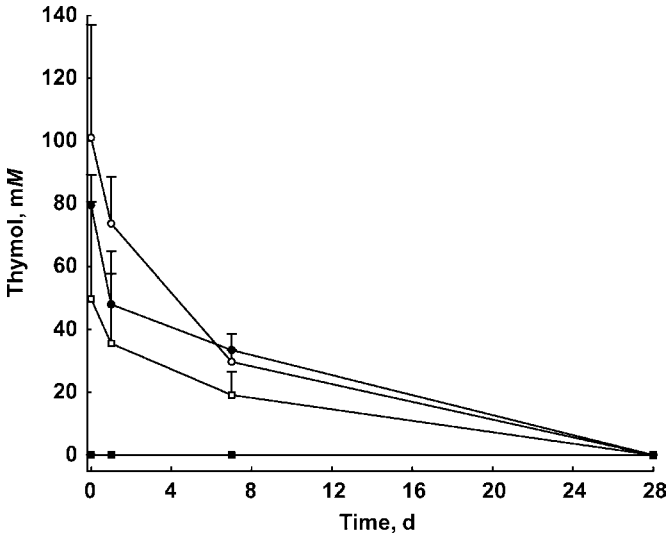

Figure 1. Disappearance of thymol from feedlot pen surfaces after thymol was topically applied and no cattle were in the pens. Treatments were: 0 m*M* —■—, 50 m*M* —□—, 80 m*M* —●—, or 100 m*M* —○—.

Pooled samples collected from the second experiment were processed as follows. A 20-g subsample was placed in 20 mL of distilled H_2O, shaken vigorously for 30 s, and pH was measured. A 15-g subsample was used to determine DM. Another 15-g subsample was acidified with 15 mL of 0.5 *M* H_2SO_4 and stored at –20°C until analyzed for thymol (as indicated earlier). Then VFA, branched VFA, and aromatic compounds (cresol, indole, skatole, 4-ethylphenol, and phenol) were analyzed using a Hewlett Packard 6890 GC as previously described

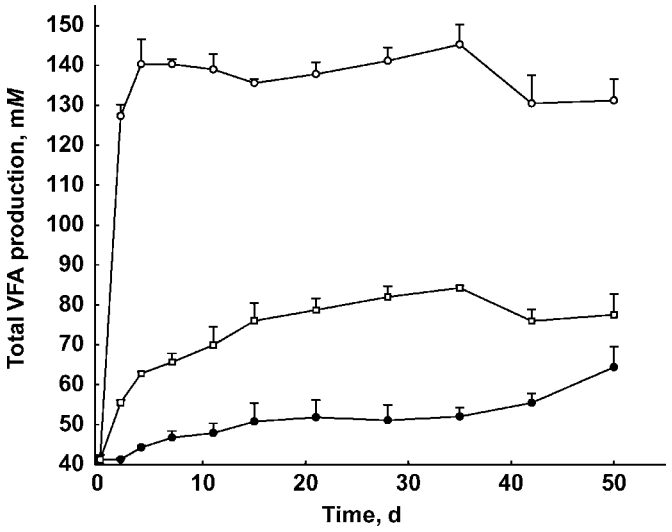

Figure 2. Accumulation of VFA in laboratory vessels containing cattle manure treated with 20 m*M* of thymol or 20 m*M* of thymol incorporated into corncob granules. Treatments were: no additions —○—, thymol in corncobs —□—, or thymol —●—.

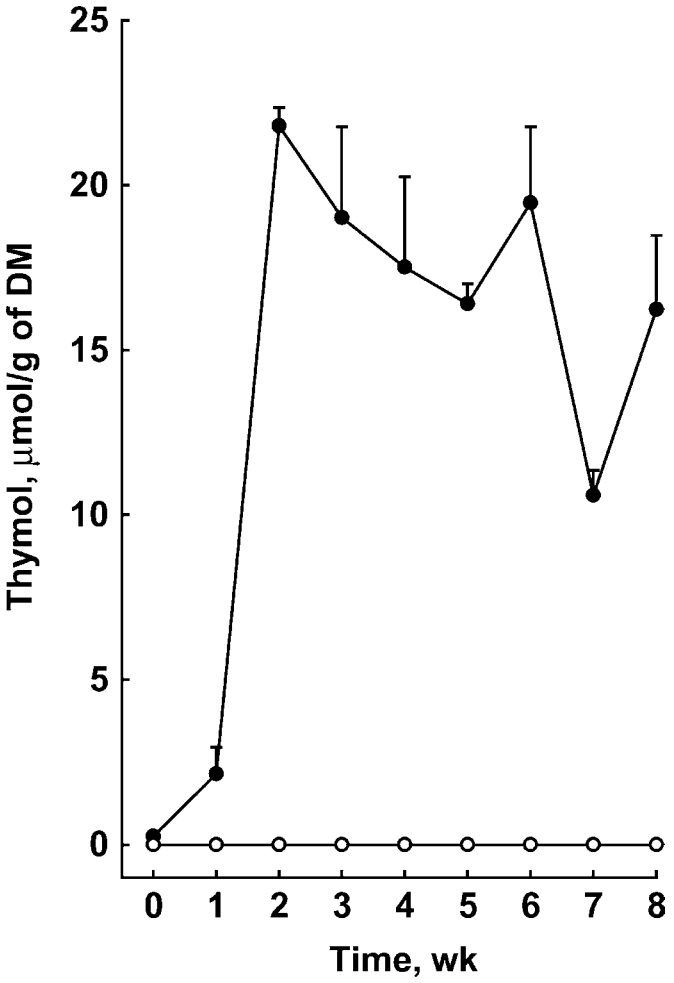

Figure 3. Concentration of thymol in manure samples collected from feedlot pens with no treatment or treated with thymol in corncobs. Treatments were: no treatment —○— or thymol in corncobs —●—.

(Miller and Varel, 2002). Total coliforms and *E. coli* were enumerated with 3M Petrifilm *Escherichia coli* coliform count plates (3M Microbiology Products, St. Paul, MN) as previously described (Varel and Miller, 2001). The presence of *E. coli* O157 in the feedlot manure was determined using the nonselective enrichment and immunomagnetic separation procedures described by Berry and Miller (2005). Presumptive *E. coli* O157 isolates were confirmed as *E. coli* by standard biochemical testing (Hitchins et al., 1998) and as *E. coli* O157 by PCR for the presence of enterohemorrhagic *E. coli* genes stx_1, stx_2, *eaeA*, EHEC *hlyA (ehxA)*, and $rfbE_{0157}$ (Paton and Paton, 1998).

Statistical Analyses

Data from the granule feedlot study were averaged before granule application (wk 0) and after application for each week in each pen, and were analyzed as a split-plot in time with the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Differences between means were tested

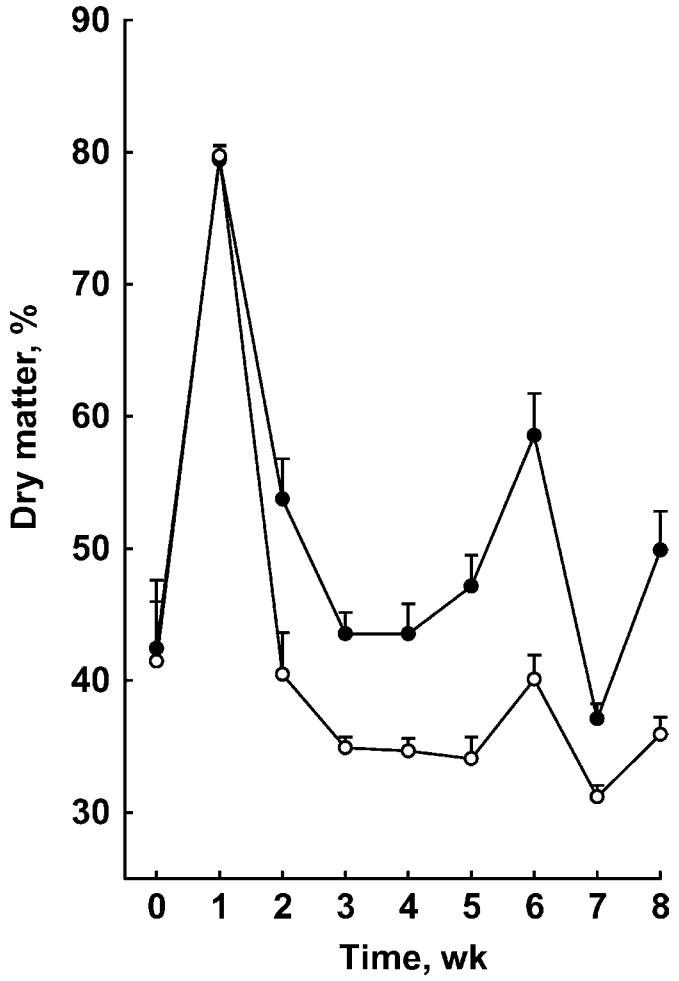

Figure 4. Percent DM of manure samples collected from feedlot pens with no treatment or treated with thymol in corncobs. Treatments were: no treatment —○— or thymol in corncobs —●—.

with a linear model that included treatment and week as discrete effects. The model was treatment, pen nested within treatment, week, and treatment × week. Treatment means were tested with pen nested within treatment as the source of error. Week and treatment × week means were tested using the residual mean squares as the source of error.

RESULTS

In the first study, concentrations of 50, 80, and 100 m*M* of thymol were applied to the feedlot surface (Figure 1). After d 1, concentrations were 37, 48, and 75 m*M*, respectively. At d 7, a further reduction of thymol was observed, and average concentrations were 21, 38, and 34 m*M*, respectively. After 28 d, no thymol was detected in any of the pens.

The laboratory study conducted with thyme oil (2.5% thymol) incorporated into corncob granules indicated that thymol without the granule was numerically more effective in inhibiting the production of VFA than thy-

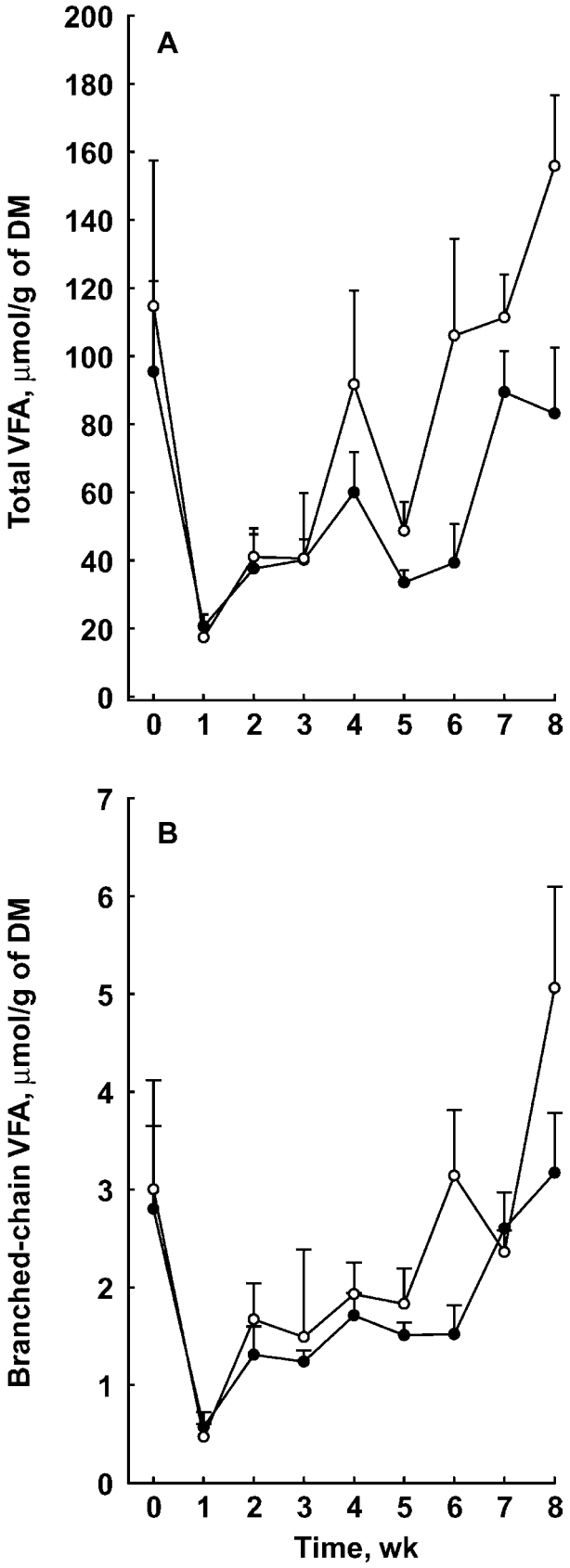

Figure 5. Accumulation of VFA and branched-chain VFA in manure samples collected from feedlot pens with no treatment or treated with thymol in corncobs. Treatments were: no treatment —○— or thymol in corncobs —●—.

Table 1. Rate of accumulation of odor compounds in feedlot manure with no treatment or treated with thymol in corncobs over an 8-wk period[1]

	Rate (μmol·g DM^{-1}·wk^{-1})		
Odor group	No treatment	Thymol	*P*-value
Total VFA[2]	18.0 ± 2.1	7.5 ± 1.3	0.01
Branched-chain VFA[3]	0.55 ± 0.06	0.31 ± 0.04	0.01
Total aromatic compounds[4]	0.065 ± 0.017	0.027 ± 0.023	0.17

[1]Means ± SEM represent n = 24 per treatment group.
[2]Includes acetate, propionate, butyrate, valerate, and caproate.
[3]Includes isobutyrate, isovalerate, and isocaproate.
[4]Includes cresol, indole, skatole, 4-ethylphenol, and phenol.

mol incorporated into the corncob granule (Figure 2, d 14; 50 compared with 76 m*M* of VFA). However, the granule containing thymol kept the maximum accumulated concentration of VFA below 85 m*M*, which was 45% below the concentration (145 m*M*) in the vessels with no additions at d 35.

In the feedlot study, in which thyme oil (2.5% thymol) was incorporated into corncob granules, the objective was to maintain approximately 15 to 20 μmol of thymol/g of DM in the feedlot manure. Corncobs containing thymol were applied on Monday, and feedlot manure was sampled on 3 consecutive days, Wednesday through Friday. This allowed 48 h for cattle foot traffic to mix the granules into the manure and thymol to exert an effect. With the exception of wk 1 and 7, the concentration of thymol was maintained between 15 and 20 μmol of DM/g (Figure 3). During the first week, it was difficult to estimate the initial mass of manure being treated; thus, the target concentration was less than our objective. As expected, the DM of the manure containing the corncob granules was greater ($P < 0.05$) than the untreated manure over the 8-wk period (Figure 4).

The rates of VFA and branched-chain VFA accumulation were less in pens that were treated with thymol incorporated into corncobs than in pens that were not treated (Figure 5). From wk 1 to 8, rates of VFA and branch-chain VFA accumulation were less ($P < 0.01$) in treated pens than in untreated pens. Rates of aromatic compound accumulation were numerically lower; however, the rates were not different from the untreated pens ($P = 0.17$; Table 1).

Escherichia coli was the predominant bacterial species contributing to the number of total coliforms found in manure samples. From wk 1 to 8, manure in pens treated with thymol in corncobs had fewer ($P < 0.04$) total coliforms and *E. coli* when compared with manure in untreated pens (Table 2). The numbers of total coliforms and *E. coli* were relatively stable after wk 1, with the exception of wk 7 when a spike in both populations increased in the manure treated with thymol (Figure 6). This increase during wk 7 coincides with a drop in the concentration of thymol in the manure (11 μmol per g of DM), which was less than the desired concentration of 15 to 20 μmol per g of DM (Figure 3). Throughout the study, *E. coli* O157 was recovered from feedlot manure from only one pen, which was an untreated pen at one end of the row of the 6 adjacent pens. This pen was positive for *E. coli* O157 beginning in wk 3 and for every week thereafter for the duration of the study. This pathogen was not recovered from any of the pens treated with the thymol.

DISCUSSION

The data suggest that thymol rapidly disappears from a feedlot surface when topically applied. After 7 d, more than 50% of all concentrations applied had volatilized or degraded. After 28 d, no thymol could be detected in any of the samples. Because thymol was applied topically, no mixing into the manure occurred, and because the manure DM was >75%, it is assumed that most of the thymol simply volatilized from the surface. When thymol is mixed with manure slurries and anaerobic conditions prevail, it is very stable (Varel, 2002). It appears to attach to organic matter, and the anaerobic microorganisms are unable to hydrolyze the phenolic ring. However, under aerobic conditions, Vokou and Liotiri (1999) concluded that essential oils can be used as carbon and energy sources by soil microorganisms, and thus, the oils would not accumulate in soil. Other experiments in which we topically applied thymol when cattle were present in the pens indicated a rapid loss of the oil (data not shown). Even with weekly topical applications of thymol, it was not possible to build up a residual concentration that was effective in reducing coliforms and odor.

The laboratory study with thymol (from thyme oil) incorporated into corncob granules indicated enough

Table 2. Average number of coliforms and *Escherichia coli* in feedlot manure with no treatment or treated with thymol in corncobs from wk 1 to 8[1]

	cfu × 10^5 per g of DM		
Bacterial group	No treatment	Thymol	*P*-value
E. coli	31.1 ± 4.0	2.9 ± 1.2	0.04
Coliforms	35.3 ± 4.2	3.7 ± 1.3	0.04

[1]Means ± SEM represent n = 24 per treatment group.

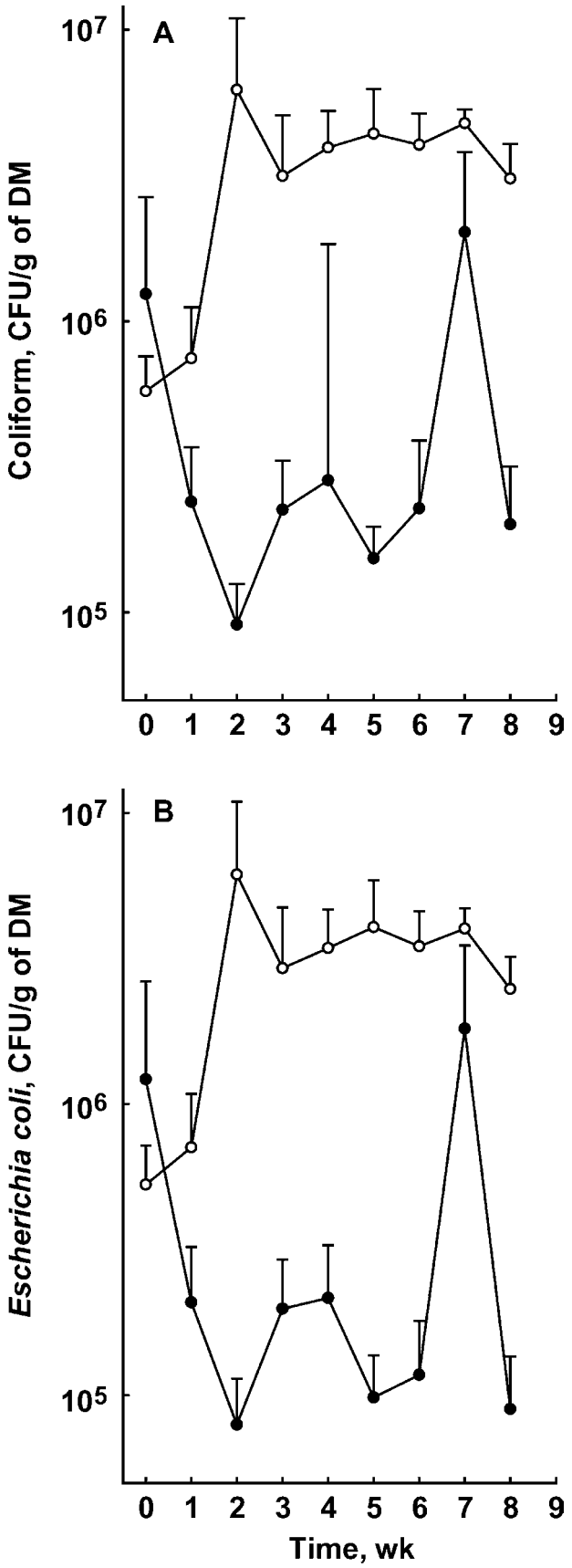

Figure 6. Number of total coliforms and *Escherichia coli* in manure samples collected from feedlot pens with no treatment or treated with thymol in corncobs. Treatments were: no treatment —○— or thymol in corncobs —●—.

thymol was released in the first few days to inhibit the fermentation activity in cattle manure (Figure 2). Apparently, not all of the thymol (20 m*M*) was released from the corncobs and available for microbial inhibition, because thymol treatment (20 m*M*) not incorporated into corncobs and added directly to the slurry was numerically more inhibitory to VFA production.

The thymol concentration (2 μmol/g of DM) during the first week after application of the corncobs containing thymol was much lower than our target of 15 to 20 μmol/g of DM (Figure 3). Because of the low thymol concentration and high manure DM (Figure 4), no difference was observed in the concentrations of VFA, branched-chain VFA, or the number of total coliforms or *E. coli* in the treated or untreated manure samples during the first week. This effect was also observed during wk 7 when the concentration of thymol decreased to 11 μmol of DM/g; a spike in total coliforms and *E. coli* culturable cells was observed (Figure 6), and an increase in the concentration of VFA and branched-chain VFA occurred (Figure 5). Although our laboratory studies indicate 10 m*M* of thymol will eradicate coliforms and *E. coli* in manure slurries (Varel and Miller, 2004), higher concentrations may not completely eradicate these pathogens in feedlot manure. This is because there is a continuous resupply of these microorganisms from feces being deposited and also because some fecal contents being sampled may not have been exposed to thymol. However, our objective to reduce coliforms in the manure was achieved. *E. coli* O157 was not recovered from any of the treated pens and from only one untreated pen. This precludes drawing any firm conclusions about the control or reduction of *E. coli* O157 by thymol treatment of feedlot manure. However, because *E. coli* O157 persisted for several weeks in the one untreated pen and was not recovered from any of the other 5 pens, it is interesting to speculate that thymol treatment may have inhibited the transmission of this pathogen to the neighboring pen or other pens, and/or the cattle in them.

Problems associated with using corncobs as a carrier included bulkiness of the cobs for handling and the limitation of being able to incorporate only 6% thyme oil (2.5% thymol) into the corncobs. Other granules that would carry a higher concentration of an oil may eliminate the need for weekly applications. We also observed that cattle did not eat the corncobs containing thymol. This is encouraging as it would likely be an undesirable effect on the animals because thymol (400 mg/L) could potentially inhibit VFA production in the rumen as shown in in vitro fermentations with mixed ruminal microorganisms (Evans and Martin, 2000). However, Cardozo et al. (2004) reported that 0.22 mg of oregano/L, which is primarily thymol, had no effect on ruminal VFA concentration after 6 d of fermentation when added to dual-flow continuous culture fermenters.

IMPLICATIONS

These studies suggest that it is possible to incorporate antimicrobial thyme oil into corncobs and thereby

maintain a concentration of thymol on a feedlot surface that is effective in reducing total coliforms and odor. Limitations to the usefulness of corncobs are that they have approximately a 6% carrying capacity for thyme oil (2.5% thymol), and they are bulky. Another granule that would carry a greater concentration of the oil may be more effective in reducing pathogens and odor and may require applications less than once per week, which was required with the corncobs. The cost of this method needs to be determined. However, we do not foresee the need to apply thymol granules year-round or over the entire feedlot surface. Rather, application would primarily be during conditions and on locations in the feedlot that have enough moisture and a temperature conducive for microbial activity.

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